Incorporation of the fasting free fatty acid concentration into quantitative insulin sensitivity check index improves its association with insulin sensitivity in adults, but not in children

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Abstract

Objective: Based on fasting insulin and glucose, several indices of insulin sensitivity have been developed in adults. Recently, it has been demonstrated that incorporation of the fasting free fatty acid (FFA) concentration improves the association with insulin sensitivity in adults. We investigated the association of clamp-derived insulin sensitivity with indices of insulin sensitivity derived from fasting blood in prepubertal children and adults, with and without incorporation of FFAs.

Design and methods: We studied 59 healthy adults and 29 of them are prepubertal children. We measured insulin sensitivity with the euglycemic–hyperinsulinemic clamp. Based on fasting insulin and glucose, we estimated insulin sensitivity with the homeostasis model assessment (HOMA), the quantitative insulin sensitivity check index (QUICKI), and the revised QUICKI after the incorporation of FFAs.

Results: The associations of HOMA and QUICKI with clamp-derived insulin sensitivity in children (rZK0.55 and 0.54 respectively; P<0.01) were similar to those in adults (rZK0.54 and 0.53 respectively; P<0.01). However, incorporation of FFAs into the QUICKI model resulted in an increase in the association in adults, but not in children (r=0.68 and 0.48 respectively; P<0.01). Adding FFA levels to a regression model with glucose and insulin as independent variables resulted in an increase in the explained variance in clamp-derived insulin sensitivity in adults, but not in children (P value 0.004 in adults and 0.3 in children).

Conclusions: HOMA and QUICKI are associated with clamp-derived insulin sensitivity in both children and adults. Incorporating fasting levels of FFAs into the QUICKI model improves the association with clamp-derived insulin sensitivity in adults, but not in children.

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Introduction

Insulin resistance is a key component of obesity, type 2 diabetes, hypertension, dyslipidemia, and subsequent cardiovascular disease (1). Recently, many studies have suggested that these disorders originate in early childhood (2). Therefore, accurate methods to measure insulin sensitivity in childhood are important to assess the presence and extent of insulin resistance, progression over time, and the effect of interventions. The gold standard technique for measurement of insulin resistance is the euglycemic–hyperinsulinemic clamp, because it directly measures the effects of insulin to promote glucose utilization under steady-state conditions (3). However, this technique is invasive and time-consuming, and therefore, difficult to perform in large groups or clinical practice, particularly in young children.

Based on the information that can be obtained from fasting blood, several indices of insulin sensitivity have been developed in adults. The simplest indexes are derived from fasting levels of insulin and/or glucose, and include fasting insulin level, the homeostasis model assessment (HOMA) (4), and the quantitative insulin sensitivity check index (QUICKI) (5). In adults, the association between these indices and insulin sensitivity measured with the euglycemic clamp has been investigated in several studies, in which correlations ranged from 0.5 to 0.8 (6, 7). Recently, it has been demonstrated that incorporation of the fasting free fatty acid (FFA) concentration, as an additional marker of insulin action, improves the association of QUICKI with insulin sensitivity in adults (‘revised QUICKI’) (8–10). Data on the validity of these indices in children using the gold standard clamp technique are scarce and show...
conflicting results (11, 12). The association between insulin sensitivity measured with the euglycemic-hyperinsulinemic clamp and revised QUICKI has not been investigated in children.

To examine these issues, we investigated the association of clamp-derived insulin sensitivity with several indices of insulin sensitivity derived from fasting blood in a group of 29 prepubertal children. In addition, we investigated these associations in 59 of their parents.

Subjects and methods

Study population
This study is part of a larger project in which vascular and metabolic variables were studied in healthy prepubertal children (13, 14) and their healthy parents (14). They were recruited as described previously (13, 14). The children had been born at the VU University Medical Center in Amsterdam. Families still living at the same address were contacted by letter and phone. All family members were of Caucasian origin. After the exclusion of one adult with non-insulin-dependent diabetes mellitus and four individuals (two children and two adults) in which one of the measurements was not performed due to logistical reasons, 59 healthy adults and 29 children were included in this study. All children were prepubertal, i.e., in Tanner stage 1. The investigation conforms with the principles outlined in the Declaration of Helsinki. The study protocol was approved by the local ethical committee. Informed consent was obtained from each adult individual. For the children, written informed consent was obtained from both parents and verbal informed consent was obtained from the children.

Measurements
Sensitivity to insulin-mediated glucose uptake was assessed by the hyperinsulinemic, euglycemic clamp technique, as described previously (13, 14). Briefly, insulin (Velosulin; Novo Nordisk, Bagsvaerd, Denmark) was infused in a primed continuous manner at a rate of 20 mU/kg per hour for 2 h. Normoglycemia (plasma glucose 5.0 mmol/l) was maintained by adjusting the rate of 20\% d-glucose infusion based on plasma glucose measurements performed at 5-min intervals. Whole body glucose uptake (M) was calculated from the glucose infusion rate during the last 60 min and expressed per unit of plasma insulin concentration (M/I) (15). Plasma insulin concentrations were measured by RIA techniques (IRMA, Medgenix Diagnostics, Fleurus, Belgium). For convenience, the M/I ratio was multiplied by 100. Children were informed extensively of what was going to happen and cartoons were created to explain the tests to the children. We performed the clamp in the child together with the clamp in one of the parents in the same room at the same time. The clamp technique was tolerated well by all parents and children. Fasting plasma glucose was measured with the hexokinase method (Roche Diagnostics). The intra- and interassay coefficients of variation (CV) for plasma glucose is 1.4 and 0.9\% respectively. Blood samples for FFA measurements were collected in prechilled tubes. Plasma was immediately separated from cells by centrifugation and frozen (−20 °C) until analysis. Plasma FFA concentrations were determined by an enzymatic colorimetric method (ELAN; Merck, Darmstadt, Germany) (16). The intra- and interassay CV of these measurements were less than 12\%, as established in our laboratory. As indices of insulin sensitivity, we used fasting insulin level, the fasting glucose-to-insulin ratio, the HOMA, the quantitative insulin sensitivity index (QUICKI) and the revised QUICKI. The glucose-to-insulin ratio was calculated by dividing fasting glucose (mmol/l) by fasting insulin (μU/ml). HOMA insulin sensitivity was calculated as fasting insulin (μU/ml) × fasting glucose (mmol/l)/22.5 (4). The QUICKI insulin sensitivity was calculated as 1/(log fasting insulin (μU/ml) + log fasting glucose (mg/dl)) (5). The revised QUICKI insulin sensitivity was calculated as 1/(log fasting insulin (μU/ml) + log fasting glucose (mg/dl) + log fasting FFA (mmol/l)) (9). Anthropometric measurements (which included weight, height, waist circumference, and hip circumference) were performed on all participants by one trained investigator (JJV) as described previously (17). The body mass index (BMI) was calculated by dividing weight in kilograms by height in meters squared. The waist-to-hip ratio was calculated as a measure of body fat distribution. Ambulatory monitoring (Spacelabs 90207) was used to obtain the 24-h recordings of blood pressure and heart rate as described previously (13, 14).

Statistical analysis
Variables are presented as mean ± s.d., or, in case of a skewed distribution, as median and interquartile range (IQR). For analyses, skewed data were log transformed. Pearson correlation analyses were used to investigate the relationships of indices of insulin sensitivity based on fasting blood samples with clamp-derived insulin sensitivity. An interaction analysis was performed to investigate whether these associations were different between adults and children. Linear regression analyses were used to investigate the association between the indices and clamp-derived insulin sensitivity. In an additional analysis, we added glucose and FFA levels to the model to investigate their contribution to the prediction of clamp-derived insulin sensitivity (adjusted R²). A two-tailed P<0.05 was considered significant. All analyses were performed using the statistical software package SPSS version 12.0 (SPSS, Chicago, IL, USA).
Results

Characteristics of the individuals are summarized in Table 1. BMI of the children ranged from 12.8 to 23.5 kg/m². Three of the children had a BMI above 20 kg/m². Measured insulin sensitivity (M/I value) was significantly lower in adults when compared with children (P<0.001). Insulin resistance as estimated by QUICKI was higher in adults and HOMA was lower in adults when compared with children, however, these differences were not statistically significant (P=0.12 and 0.15 respectively). FFA levels were significantly higher in children when compared with adults (P=0.03). The revised QUICKI was similar in parents and children (P=0.5).

The hyperinsulinemic–euglycemic clamp was performed with glucose concentrations of 5.0±0.2 and 5.0±0.1 in adults and children respectively. Insulin levels were increased to 485 pmol/l (IQR 439–581) in adults and 302 pmol/l (IQR 244–338) in children.

**Correlations between indices of insulin sensitivity derived from fasting blood and clamp-derived insulin sensitivity in children and adults**

The correlations between the indices of insulin sensitivity derived from fasting blood and clamp-derived insulin sensitivity are shown in Table 2. These associations were similar in adults and children. However, incorporation of FFA into the QUICKI model resulted in an increase in the association in adults, but not in children. The associations of clamp-derived insulin sensitivity with fasting insulin, HOMA and Revised QUICKI were similar in children and adults (P for interaction 0.6), whereas the association of clamp-derived insulin sensitivity with revised QUICKI was stronger in adults when compared with children (P for interaction 0.09). Scatter plots of the associations of clamp-derived insulin sensitivity with QUICKI and revised QUICKI in children and adults are shown in Fig. 1. Gender did not have a significant effect on the relationship between the indices of insulin sensitivity and clamp-derived insulin sensitivity.

**Associations between individual components of the insulin sensitivity indices and clamp-derived insulin sensitivity in children and adults**

Subsequent analyses demonstrated that FFA levels were associated with insulin sensitivity in adults, but not in children (Table 3). This association was different between adults and children (P for interaction 0.1). Multivariate analyses are shown in Table 4. Fasting insulin was associated with insulin sensitivity in both adults and children (Model 1). Model 2 demonstrates that glucose is not independently associated with clamp-derived insulin sensitivity. In both adults and children, adding glucose did not change the adjusted R² of the model. In adults, but not in children, FFA levels were independently associated with insulin sensitivity (Model 3). Adding FFA levels to the model resulted in a significant increase in the R² in adults, but not in children (P value for change in R² is 0.004 in adults and 0.3 in children).

**Discussion**

This study shows that simple indices of insulin sensitivity derived from fasting blood levels of insulin and glucose were associated with clamp-derived insulin sensitivity in children. In addition, these associations in children were similar to those in adults. Importantly, we report that adding fasting levels of FFAs to the QUICKI model improved the association with clamp-derived insulin sensitivity in adults, but not in children.
In adults, the association between the HOMA and QUICKI on the one hand and insulin sensitivity measured with the euglycemic clamp on the other hand has been investigated in several studies, and these indices are widely used as a measure of insulin sensitivity (6). The associations in our adult individuals were similar to those reported in previous studies in adults. In addition, the associations of the HOMA and QUICKI with clamp-derived insulin sensitivity in prepubertal children were remarkably similar to those in adults. Therefore, these indices may be of similar value in children and adults.

To our knowledge, only two previous studies have investigated the association between indices of insulin sensitivity derived from fasting blood and clamp-derived insulin sensitivity in children. Recently, in a mixed cohort of black and white children and adolescents, Gungor et al. (11) demonstrated a very high correlation between fasting indices of insulin sensitivity (fasting insulin, HOMA, QUICKI, the ratio of fasting glucose to fasting insulin) on the one hand and insulin sensitivity measured with the euglycemic clamp on the other hand (correlations approximately 0.9). In contrast, much weaker associations were found in a group of obese children (correlations ranged from 0.4 to 0.7 with different indices of insulin resistance, i.e., HOMA, QUICKI, and fasting glucose-to-insulin ratio) (12). Our findings are in accordance with the second study, but seem to be in contrast with the first. However, subgroup analyses in the study of Gungor et al. (11) demonstrated that in prepubertal Caucasian children correlations ranged from 0.54 to 0.63, which is similar to those in our study of prepubertal Caucasian children.

Several other studies have investigated the association between indices of insulin sensitivity with insulin sensitivity as estimated from the frequently sampled i.v. glucose tolerance test (18, 19). However, the frequently sampled i.v. glucose tolerance test is only an indirect estimate of insulin sensitivity that is obtained by fitting i.v. glucose tolerance data to a mathematical model with several well-documented errors (20).

In both adults and children, the association of clamp-derived insulin sensitivity with fasting insulin levels was similar to its association with HOMA and QUICKI. The presence of glucose in the HOMA and QUICKI indexes clearly did not increase the strength of the association with clamp-derived insulin sensitivity, which is a reflection of the observed weak association between glucose levels and insulin sensitivity. This is in line with previous studies (11, 21–23) and probably explained by the absence of diabetic individuals in our study and the relatively small range of glucose values.

Although the associations of QUICKI and HOMA with insulin sensitivity were similar in adults and children, it should be emphasized that the indices of insulin sensitivity are only a surrogate measure of clamp-derived insulin sensitivity. The simple indices of insulin sensitivity only explain 28–34% of the variation in clamp-derived insulin sensitivity. Although the explained variance could be increased to 46% by incorporating FFA levels into QUICKI in adults, more than half of the variance in clamp-derived insulin sensitivity is still explained by other factors. Therefore, if the study groups are small or precise changes in insulin sensitivity are sought, the hyperinsulinemic–euglycemic clamp should be used. Furthermore, it should be realized that from a pathophysiological standpoint, the indices of insulin sensitivity that are based on fasting measurements (hepatic insulin sensitivity) and clamp-derived insulin sensitivity (peripheral insulin sensitivity) provide different information (6).

Several previous studies in adults demonstrated that incorporating serum FFA levels into the QUICKI

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**Table 3** Correlations of fasting blood measurements of insulin, glucose, and fasting free fatty acid (FFA) levels with clamp-derived insulin sensitivity.

<table>
<thead>
<tr>
<th>Clamp-derived insulin sensitivity (M/I value)</th>
<th>Children</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin level</td>
<td>-0.58*</td>
<td>-0.58*</td>
</tr>
<tr>
<td>Glucose level</td>
<td>-0.03</td>
<td>0.05</td>
</tr>
<tr>
<td>FFA level</td>
<td>0.002</td>
<td>-0.41*</td>
</tr>
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</table>

*P<0.01.
improves its association with insulin sensitivity measured with the clamp (8–10). To investigate whether the effect of the incorporation of FFAs also holds true for children, we first performed the current analyses in children only. In the children, we observed that the incorporation of FFAs into the QUICKI model did not result in any improvement of its association with clamp-derived insulin sensitivity. In addition, FFA levels were not associated with insulin sensitivity. These were unexpected findings. As a control, we analyzed the data in the parents. In line with previous findings in adults, the incorporation of FFAs into QUICKI resulted in a significant improvement of its association with insulin sensitivity, and FFA levels were associated with insulin sensitivity. These findings in the parents suggest that the absence of an effect in children is not due to the factors related to general measurement errors, the selection of the study population, and/or factors related to the investigators.

In our study, FFA levels in children (0.67 ± 0.3 mmol/l) were somewhat higher than those in adults (0.55 ± 0.2 mmol/l, \( P = 0.03 \)). We could not find another report that compared adults and children. However, the normal range of FFA levels is around 0.2–1.2 mmol/l. In addition, in the previous studies in children, FFA levels ranged from 0.3 to 0.9 mmol/l (24–28), which is in accordance with our findings.

It could be argued that the absence of an effect of FFA levels in children may be due to the limited size of the population. However, the absence of an association between FFA levels and insulin sensitivity is in line with two previous studies in children. These studies demonstrated that FFA levels were not associated with insulin sensitivity as estimated from fasting insulin and glucose levels (24–26). In addition, it should be pointed out that we did not observe any improvement in the association between QUICKI and clamp-derived insulin sensitivity after the incorporation of FFA levels. In the parents, however, we observed a significant improvement, and the difference between parents and children was significant. Therefore, these observed differences between parents and children may suggest differences in insulin-mediated effects on lipolysis in children when compared with adults. The precise mechanisms, however, remain obscure.

In conclusion, methods to measure insulin sensitivity in childhood are important to assess the presence and extent of insulin resistance, progression over time, and the effect of interventions. We demonstrate that simple indices of insulin sensitivity derived from fasting blood levels of insulin and/or glucose were associated with clamp-derived insulin sensitivity in children and adults. However, the explained variance of clamp-derived insulin sensitivity is disappointing. Importantly, incorporating fasting levels of FFAs into the QUICKI model improves the association with clamp-derived insulin sensitivity in adults, but not in children. Further research is necessary to develop better estimates of insulin sensitivity in childhood.

Declaration of interest
The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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References

Table 4 Multivariate analyses of the association between individual components of the insulin sensitivity indices and clamp-derived insulin sensitivity in children and adults.

<table>
<thead>
<tr>
<th></th>
<th>Children</th>
<th></th>
<th>Adults</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standardized ( \beta )</td>
<td>( P ) value</td>
<td>Adjusted ( R^2 )</td>
<td>Standardized ( \beta )</td>
</tr>
<tr>
<td>Model 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Insulin level</td>
<td>-0.58</td>
<td>0.001</td>
<td>0.31</td>
<td>-0.58</td>
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<td>Model 2</td>
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<td>-0.59</td>
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<tr>
<td>Model 3</td>
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<td>Glucose level</td>
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<td>0.001</td>
</tr>
<tr>
<td>FFA level</td>
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<td>0.3</td>
<td>0.33</td>
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